Duration of human papillomavirus persistence and its relationship with recurrent cervical dysplasia

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Objective To evaluate how the duration of human papillomavirus (HPV) persistence influences the risk of developing recurrent high-grade cervical dysplasia (CIN2+).

Methods Data of patients with persistent HPV infection (at least at 6 months) after primary conization were extracted from a multi-institutional Italian database, retrospectively. Kaplan–Meier and Cox proportional hazards models were used to evaluate associations between duration of HPV persistence with the 5-year risk of developing recurrent CIN2+.

Results Overall, 545 patients met the inclusion criteria. Positive margins were detected in 160 (29.3%) patients. Overall, 247 (45.3%) and 123 (22.6%) patients had a documented infection from HPV16/18, and other high-risk HPV types. 187 (34.3%), 73 (13.4%), and 40 (7.3%) were diagnosed with persistent HPV infection at 12, 18, and 24 months, respectively. Patients with HPV persistence at 6 months experienced a risk of recurrence of 7.46%. Twelve-month HPV persistence strongly correlates with the risk of developing the recurrent disease (risk of recurrence: 13.1%). While, having HPV persistence >12 months did not correlate with an increased risk of recurrence (hazard ratio: 1.34 (95% confidence interval: 0.78–2.32); $P = 0.336$, log-rank test).

Conclusion HPV persistence is one of the most important factors predicting the risk of CIN2+ recurrence. The risk of CIN2+ recurrence increased with the increase of HPV persistence for up to 1 year. The persistence of HPV after the first year does not appear as a risk factor. *European Journal of Cancer Prevention* 32: 525–532

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Introduction

Human papillomavirus (HPV) is a double-stranded DNA virus that might infect basal cells of stratified epithelia in humans. Most HPV infections revolve spontaneously, within 1 or 2 years (Falcaro *et al.*, 2021; Bukowski *et al.*, 2022; Smith *et al.*, 2022). Persistent HPV infection might cause precancerous and cancerous lesions in various districts (including the lower genital tract and the head and neck area). In particular, nearly all cervical dysplasia and cervical cancer are related to HPV. About 70% of cervical lesions are due to HPV16 and HPV18 (Demarco *et al.*, 2022); however, other high-risk HPV types might determine the occurrence of lesions of the uterine cervix (Falcaro *et al.*, 2021; Bukowski *et al.*, 2022; Demarco *et al.*, 2022; Mix *et al.*, 2022; Smith *et al.*, 2022).

The role of HPV in determining the occurrence of cervical dysplasia and cervical cancer is well established (Falcaro *et al.*, 2021; Bukowski *et al.*, 2022; Demarco *et al.*, 2022; Mix *et al.*, 2022; Smith *et al.*, 2022). More interestingly, HPV persistence, as well as viral load, are considered two of the main risk factors impacting the risk of cervical dysplasia recurrence (Bogani *et al.*, 2021b; Cicconi *et al.*, 2022;

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Prétet *et al*., 2022). Other main risk factors influencing the risk of cervical dysplasia recurrence include patients' age, positive margin status, and severity of the lesions (Bogani *et al*., 2019, 2020a, 2021b).

Recurrent cervical dysplasia might have a great impact on patients' journeys. Patients experiencing persistent or recurrent cervical dysplasia are at high risk of developing invasive lesions of the uterine cervix, as well as developing treatment-related morbidity of another therapeutic approach. Secondary conization correlates with fertility, obstetrical, surgical, and oncological issues. In particular, in young women, the excessive removal of the cervical tissues is associated with an increased risk of developing preterm delivery (Firichenko *et al*., 2021). This latter condition is heavily impacting women and newborns. For all those reasons several studies tried to identify factors predicting the risk of cervical dysplasia persistence or recurrence (Bogani *et al*., 2020b, 2022). Accumulating data suggested that HPV persistence after conization increased this risk by about three-fold, being HPV persistence the most impacting risk factor on the risk of developing persistent or recurrent cervical dysplasia (Bogani *et al*., 2019, 2020b, 2022; Firichenko *et al*., 2021); however, correlating the impact of the duration of HPV persistence with the risk of developing recurrent cervical dysplasia are scant. Although few data support that long-term HPV persistence is a risk factor for developing cervical lesions, no studies investigated whether long-term HPV persistence after conization is a real risk factor for recurrent cervical dysplasia. Moreover, there is no consensus regarding the definition of long-term persistence. In the present paper, we aimed to investigate outcomes of women with persistent HPV infection 6 months after primary conization. Additionally, we sought to determine a cutoff on how the duration of HPV persistence impacted patients' outcomes.

Materials and methods

This is a retrospective multi-institutional study conducted in Italy. Institutional Review Board (IRB) approval was obtained (IRB#6812). For the present study, we collected charts of patients with newly diagnosed high-grade cervical dysplasia (HSIL/CIN2+) treated in Italy from 1 January 2010 to 31 December 2014. Data of the patients included in the present study are included in a dedicated dataset (available upon request). Overall, 32 centers in Italy participated in this multi-institutional study (Supplemental material 1, Supplemental digital content 1, *http://links.lww.com/EJCP/A402*).

The inclusion criteria were: patient's age 18 or more; having the first conization due to HSIL/CIN2+; squamous cell lesions; diagnosis of HPV persistence after primary conization (regardless of the HPV types); data of follow-up at 5 years. Exclusion criteria were: ongoing pregnancy; consent withdrawal; glandular lesions; lack of data about 5-year follow-up; previous hysterectomy; history of other cervical excision due to HSIL/CIN2+; diagnosis of invasive carcinoma; history of HIV or other conditions influencing the immune response.

Demographic details, data about HPV type(s) detected, as well as data on treatment for the occurrence of cervical dysplasia were retrospectively reviewed. HPV types were considered high-risk according to the data of the International Agency for Research on Cancer (IARC) (IARC working group, 2020). Data of a part of the patients included in this research is already included in other researches published by our study's group (Bogani *et al*., 2020a, 2020b, 2021b, 2022); however, previous investigations were focused on other features, and the findings reported in this study are not duplicated (Bogani *et al*., 2020a, 2020b, 2021b, 2022).

Given the multi-institutional nature of the study, different surgeons performed primary conization in various centers, across the whole study period. No differences in the facilities available for patients' care and the referral patterns of various services were consistent over the study period. Details regarding the type of preoperative diagnosis, treatment, follow-up schedule, and clinical visits are described in the previous investigation of our study group (Bogani *et al*., 2020b, 2022). Briefly, patients included in the study received cervical conization due to the diagnosis of cervical dysplasia. Preoperative biopsy or definitive histology confirmed the presence of HSIL/CIN2+ in all cases. Generally, cervical conization was performed under local anesthesia, in an outpatient setting. Patients were evaluated after 3 or 6 months after conization in case of positive or negative cervical margins, respectively. Therefore, patients were evaluated every 6 months (until 2 years), then annually (until 5 years). Clinical examination included cytology and colposcopy at the time of every evaluation.

All patients of the study population underwent HPV DNA testing at the time of the first examination. Then, HPV DNA testing was performed based on the discretion of treating physicians, generally in patients with documented high-risk HPV infection. Cervical biopsies were indicated in case of abnormal results at cytological or colposcopic examination. Persistence of HPV infection was defined as the persistence of HPV detected at the first clinical examination following conization (generally at 3 or 6 months). Generally, patients detected with a low-grade lesion (CIN1/LSIL) had observation only. While, when patients detected with high-grade lesions (HSIL/CIN2+) were submitted to another surgical treatment (mostly secondary conization or hysterectomy in selected cases). Usually, medical treatments were not proposed in patients with recurrent cervical dysplasia. All centers included followed the guidelines proposed by the Italian Society of Colposcopy and Cervico Vaginal Pathology for diagnosis, treatment, and follow-up

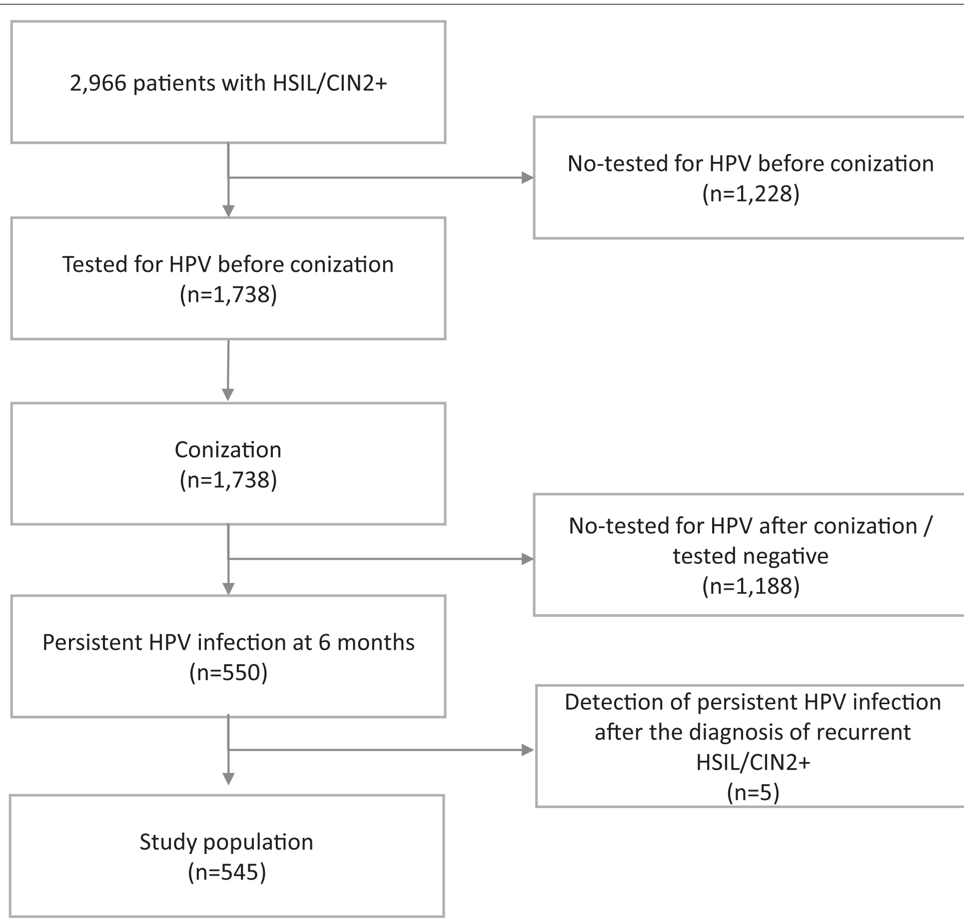
(Ciavattini *et al.*, 2022). For the purpose of this study, we adopted a hierarchical clustering of HPV types. HPV types were classified as HPV16/18, other high-risk HPV types, and unspecified (also including low-risk HPV types) (Wentzensen *et al.*, 2010). Recurrent disease was defined as the persistence or recurrence of high-grade cervical dysplasia requiring secondary conization or hysterectomy. Patients who had no secondary surgical treatment were considered without recurrence. The persistent disease was defined when recurrent HSIL/CIN2+ was observed at the time of the first examination after primary conization. Recurrent disease was defined when the patient had at least one negative examination before the diagnosis of recurrent HSIL/CIN2+.

Basic descriptive statistics were used to describe the study population. The risk of developing recurrence was evaluated using Kaplan–Meier and Cox proportional hazard regression models. Hazard ratio (HR) and 95% confidence intervals (CIs) were calculated for each comparison. Univariate and multivariate analyses were performed when appropriate. Duration of follow-up was counted from the date of the first conization and the date last follow-up or secondary conization or hysterectomy. Statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Software, San Diego California, USA), IBM-Microsoft SPSS (SPSS Statistics. International Business Machines Corporation IBM 2013 Armonk, New York, USA) version 20.0.

Results

Charts of 2966 patients who had primary treatment for HSIL/CIN2+ were evaluated. We selected cases of all patients with persistent HPV infection at 6 months after primary conization. Overall, 550 (18.5%) patients with persistent HPV infection (at 6 months after primary conization) were identified. Five (0.2%) patients were excluded because they were diagnosed with persistent HSIL/CIN2+ before the execution of HPV testing, thus leaving 545 (18.4%) patients for the final analysis. Figure 1 shows the flow of patients through the study design.

Fig. 1



Study design.

Study population

Median (range) populations' age and BMI were 44 (20, 74) years and 22 (14.4, 42) kg/mq, respectively. Table 1 reposts details of the study population. Overall, 173 (31.7%) and 372 (68.3%) patients had primary conization due to CIN2 and CIN3, respectively. Positive margins were detected in 160 (29.3%) patients: 117 (21.4%), 41 (7.5%), and 1 (0.2%) patients had positive ectocervical, endocervical, and ectocervical plus endocervical margins, respectively. Overall, 247 (45.3%) and 123 (22.6%) patients had a documented infection from HPV16/18, and other high-risk HPV types. Unspecified HPV infection (including also low-risk HPV types) accounted for 174 (31.9%) patients. Overall all patients included had HPV persistence at 6 months. Moreover, 187 (34.3%), 73 (13.4%), and 40 (7.3%) were diagnosed with persistent HPV infection at 12, 18, and 24 months, respectively. HPV16/18 and other high-risk HPV types are more likely to persist longer than unspecified/low-risk HPV types ($P = 0.029$). Table 2 reports details regarding the crude number of recurrences observed among patients with persistent HPV infection.

Table 1 Main characteristics of the study population

Characteristics	$N = 545$
Age, years	44 (20, 74)
BMI, kg/mq	22 (14.4, 42)
Reason for primary conization	
CIN2	173 (31.7%)
CIN3	372 (68.3%)
Positive margins	
Yes	160 (29.3%)
No	385 (70.7%)
Adjuvant HPV vaccination	
Yes	51 (9.4%)
No	494 (90.6%)
Type of persistent HPV	
HPV16/18	247 (45.3%)
Other high-risk HPV	123 (22.6%)
Unspecified	174 (31.9%)
Duration of HPV persistence	
6 months	545 (100%)
12 months	187 (34.3%)
18 months	73 (13.4%)
24 months	40 (7.3%)

Data are expressed in median (range) or number (%).
CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.

Table 2 Details regarding types of human papillomavirus involved at various time frames

	HPV16/18	Other high-risk HPV	Unspecified
Persistence at 6 months ($n = 545$)	247 (45.3%)	123 (22.6%)	174 (31.9%)
Persistence at 12 months ($n = 187$)	101 (54%)	44 (23.5%)	42 (22.5%)
Persistence at 18 months ($n = 73$)	38 (52.1%)	19 (26%)	16 (21.9%)
Persistence at 24 months ($n = 40$)	22 (55%)	13 (32.5%)	5 (12.5%)

Data are expressed in number (%).
HPV, human papillomavirus.

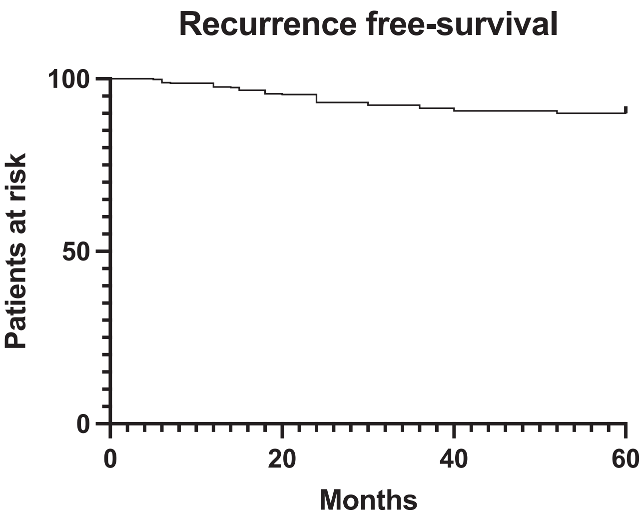
Factors associated with recurrent high-grade cervical dysplasia

Figure 2 shows the risk of developing recurrence over the five-year follow-up, for the whole population of patients. Via univariate analysis, no factor was associated with the risk of recurrence. In particular, we evaluated the impact of age, margins' status, vaccination status, and type of persistent HPV types on the risk of developing recurrent HSIL/CIN2+. In our population, increasing in age did not impact the risk of developing recurrent HSIL/CIN2 + ($P = 0.372$). Similarly, patients aged 45 years or more were not associated with a higher risk of recurrence in comparison to patients aged less than 45 years of age ($P = 0.873$, log-rank test). Overall, 160 (29.3%) patients had positive margins. The presence of positive surgical margins did not impact the risk of developing recurrent HSIL/CIN2 + ($P = 0.984$; log-rank test). Adjuvant HPV vaccination after primary conization was delivered in 51 (9.4%) patients. Patients who had vaccination experienced a trend toward lower risk of developing HSIL/CIN2+ recurrence in comparison to those who had not (HR: 0.189; 95% CI: 0.074–0.483; $P = 0.063$, log-rank test). Type of persistent HPV involved (HPV16/18, other high-risk HPV, unspecified/low-risk) was not associated with patients outcomes ($P = 0.167$, log-rank test). The 5-year risk of developing HSIL/CIN2+ recurrence according to vaccine status, margin status, and type of persistent HPV types is displayed in Fig. 3. A multivariate analysis was not performed because no factor was associated with HSIL/CIN2+ recurrence.

Duration of human papillomavirus persistence

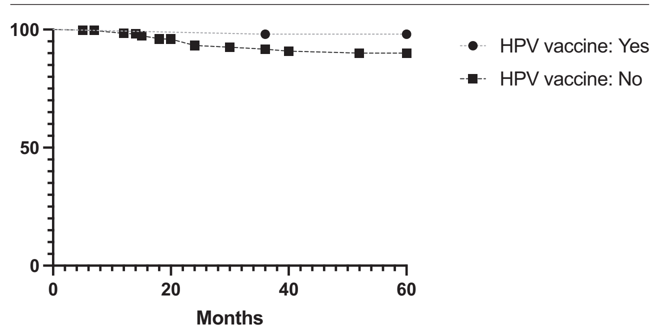
Patients with recurrent HSIL/CIN2+ had a longer duration of persistent HPV in comparison to patients with no recurrence (12.2 vs. 9.0 months; $P < 0.001$). We observed that patients with 12-month HPV persistence had a two-fold increase in recurrence rate in comparison to patients with 6-month HPV persistence; however, a prolonged persistence (more than 12 months) did not increase the risk of developing recurrence. Overall, patients with 6-month HPV persistence experienced a risk of recurrence of 7.46% over the 5-year follow-up period. Twelve-month HPV persistence strongly correlates with the risk of developing the recurrent disease (risk of recurrence over time: 13.1%). While having HPV persistence >12 months did not correlate with an increased risk of recurrence (HR: 1.34 (95% CI: 0.78–2.32); $P = 0.336$, log-rank test; Supplemental material 2, Supplemental digital content 2, *http://links.lww.com/EJCP/A403*). In comparison to HPV persistence at 12 months, HPV persistence at 18 months (risk of recurrence over time: 8.82%; $P = 0.351$) and 24 months (risk of recurrence over time: 10.53%; $P = 0.660$) was not associated with an increased risk of recurrence. Figure 4 shows the cumulative risk of recurrence for patients with persistent HPV at 12, 18, and 24 months.

Fig. 2



Five-year risk of developing recurrent cervical dysplasia in women with persistent HPV infection. HPV, human pappilomavirus.

Fig. 3



Five-year risk of developing recurrent cervical dysplasia according to age, margins' status, adjuvant HPV, and type of persistent HPV. HPV, human pappilomavirus.

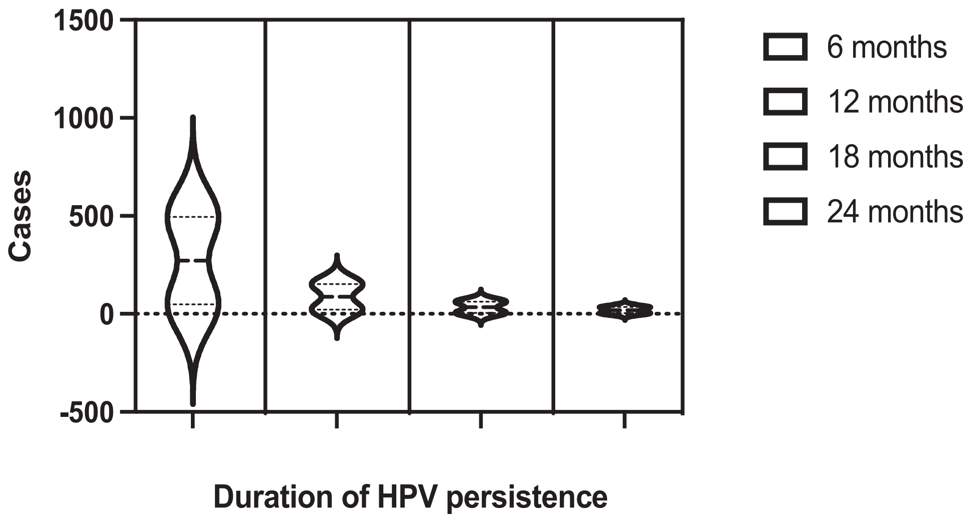
Discussion

The present study evaluated the role of HPV persistence among women undergoing conization for high-grade cervical dysplasia. HPV persistence is a well known risk factor for developing primary and recurrent cervical dysplasia. Several studies highlighted the prognostic significance of viral load and HPV persistence in women having conization; however, no data about correlating the duration of HPV persistence and patients' outcomes are available. Here, for the first time, we tried to assess how the duration of HPV persistence influences. We observed that HPV persistence at 12 months influences the risk of developing recurrent high-grade cervical dysplasia; while HPV persistence after 12 months (at 18 and 24 months) is not associated with a more pronounced increased risk.

Accumulating evidence has provided strong and consistent data that HPV persistence is the main factor influencing the risk of developing HPV-related disease, including cervical dysplasia and cervical cancer (Casarin *et al*., 2020; Falcaro *et al*., 2021; Mix *et al*., 2022; Di Donato *et al*., 2023; Giannini *et al*., 2023). Duration of HPV persistence, viral load, deregulation of HPV gene expression, and host epigenetic mechanisms (including methylation of imprinted gene control) are the main factor influencing the process of tissue transformation (Smith *et al*., 2022; Cicconi *et al*., 2022; Demarco *et al*., 2022; Mix *et al*., 2022). Although a high level of evidence highlighted that HPV persistence correlates with the risk of developing either primary or recurrent cervical dysplasia, no evidence regarding the impact of duration of HPV infection is still available. There is a logical assumption that a long-term infection correlates with a high risk of developing precancerous and cancer transformation of the epithelium. Theoretically, the longer the infection, the higher the risk of developing lesions. However, no studies evaluated this issue in patients with high-grade cervical dysplasia. Few data are available looking at patients with low-grade cervical dysplasia. Because observation is the preferred option for patients with LSIL/CIN1, data are evaluating the impact of HPV persistence in this setting (Egemen *et al*., 2020; Leung *et al*., 2020). Data from an epidemiological study on 1.5 million individuals aged from 25 to 65 years of age undergoing screening for cervical cancer, highlighted that after two consecutive diagnoses of CIN 1, about one out of two patients continued to be HPV-positive, and of those, over 90% had follow-up cytology that was LSIL or less (Egemen *et al*., 2020). More interestingly from a clinical point of view, in a study evaluating 126 patients having conization for persistent CIN1 (diagnosed and confirmed with two consecutive cervical biopsies under colposcopic guidance), 13% of patients were diagnosed with CIN2+ on the cervical specimen (Leung *et al*., 2020). These data supported that HPV persistence influences the risk of progression from low- to high-grade lesions.

Four points of the present study deserve to be discussed. First, several mechanisms of HPV clearance and persistence exist. Constitutional variables, modifiable factors (including tobacco and medication use), and epigenetic interactions are influencing HPV persistence and patients' outcomes. In the present study, we were not able to evaluate all these parameters. Second, we are not able to evaluate the duration of HPV persistence before the primary treatment. We can speculate that the duration of HPV persistence before primary conization might have a great impact on the interpretation of our results. Third, our study evaluated the outcomes within the first 5 years. We can suppose that we are missing some recurrence, especially in patients with HPV persistence longer than 12 months. However, we have to point out that almost all patients included in the present series developed recurrence within the first 2 years. This finding is

Fig. 4



The cumulative risk of recurrence for patients with persistent HPV at 12, 18, and 24 months. HPV, human pappilomavirus.

corroborated by a large body of Literature. Fourth, only a minority of patients included in this study received adjuvant vaccination. Although we are expecting a limited effect of HPV vaccination in this cluster of patients, growing evidence supports the adoption of vaccination after primary treatment (Bogani *et al*., 2021a, 2023; Di Donato *et al*., 2022). Even in our series, we observed that patients receiving vaccination were at a lower (but not statistically significant) risk of developing HSIL/CIN2+ recurrence in comparison to those who had not. Hence, further research testing the effectiveness of vaccination in patients with persistent HPV infection is needed. The main strength of the present study is its novelty. To date, to the best of our knowledge, no other studies evaluated this issue. Other strengths included the large sample size of the population of interest, the long-term follow-up period, and the collection of consecutive patients with newly diagnosed CIN2+. The inherent biases of the retrospective study design are the main weaknesses of the present research. Moreover, we are not evaluating several factors (measurable and not measurable) influencing directly HPV persistence and the risk of developing recurrent CIN2 + (Lebeau *et al*., 2022; Pan *et al*., 2022). Other main limitations included: The inclusion of both CIN2 and CIN3 lesions; they are characterized by different natures, behavior, and the different regression capacity. The evaluation of both low- and high-risk viruses, because they are related to the different risks of progression (over time). The multicenter study design might be considered a limitation; although it improves the generalization of our results, the adoption of various methods of HPV testing across the participating center might impact the interpretation of our results. Hence, we stressed that our results should be considered preliminary and have to be validated in further research on this issue.

In conclusion, the present paper evaluated how the duration of HPV persistence after primary conization impacted the risk of developing recurrent CIN2+. Our results suggested that HPV persistence at 6 and 12 months increases the 5-year risk of developing CIN2+ recurrence. The risk of developing CIN2+ recurrence remains almost stable in patients with HPV persistence longer than 12 months. Our preliminary data support dedicated counseling with the execution of HPV testing at 6 and 12 months after primary conization; while the HPV testing after 12 months seems to not provide useful data for assessing patients' prognosis. However, owing to the inherent limitations of the study design, new evidence is warranted. A further prospective study evaluating the impact of duration of HPV persistence is needed to better tailor surveillance in patients having primary treatment for cervical dysplasia.

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Conflicts of interest

There are no conflicts of interest.

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